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Thyroid gland and pregnancy

(Diploma Thesis)

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2. ABBREVIATIONS

ACOG	:	American College of Obstetricians and Gynecologists
AITD	:	Autoimmune thyroid disorder
anti-TgAb	:	anti-thyroglobulin
anti-TPO	:	anti-thyroid peroxidase
cAMP	:	cyclic adenosine monophosphate
CL	:	chorion laeve
HPTA	:	Hypothalamus-Pituitary-Thyroid-Axis
DIT	:	Diiodotyrosine
GD	:	Grave's disease
hCG	:	human chorionic gonadotropin
HT	:	Hashimoto's thyroiditis
MIT	:	Monoiodotyrosine
MMI	:	Methimazole
NIS	:	Sodium-iodine-symporter
PPTD	:	Postpartum thyroid disorder
PPT	:	Postpartum thyroiditis
PTU	:	Propylthiouracil
T3	:	Triidothyronine (T3)
T4	:	Tetraidothyronine or thyroxine
TH	:	Thyroid Hormones
TBG	:	Thyroxine-binding globulin (TBG)
TPO	:	Thyroixide Peroxidase
TRE	:	Thyroid responsive element
TRAbs	:	TSH receptor antibodies
TRH	:	Thyrotropin-Releasing-Hormone (TRH)
TSH	:	Thyroid-Stimulating-Hormone (TSH)
SYS	:	Secondary Yolk Sac
UC	:	Umbilical cord
ECC	:	Exocoleomic cavity
AC	:	Amniotic cavity
CF	:	Coleomic fluid

3. ABSTRACT ENGLISH

Thyroid physiology involves a range of biochemical processes in the human body, and is characterized by a respectively wide range of thyroid disorders. Thyroid hormones are produced by the thyroid gland, with through the sequestration of iodine as basic element. This process is regulated both intrathyroidally through enzymatic processes and extrathyroidally through the Hypothalamus-Pituitary-Thyroid-Axis (HPTA). The bioavailability of thyroid hormones and their biological activities in peripheral tissues are regulated by special enzymes called deiodinases.

Pregnancy is a state of unique physiological adaptations for both the mother and the fetus that are governed by the maternal-fetal interaction through the placenta. Thyroid hormones are vital in maternal physiology throughout gestation as well as regulators of fetal development. The increased needs in thyroid hormone production during pregnancy pose a stress on thyroid and raise the needs for iodine intake. The inability of the thyroid gland to adapt to this state is marked by increased incidence of maternal and fetal complications such as preeclampsia, preterm delivery or even fetal death or cretinism.

Such events are also observed in the case of preexisting or acquired thyroid disorders. Grave's disease and Hashimoto's thyroiditis are two of the most common thyroid disorders responsible for thyroid hormone disturbances during gestation. Immune system adaptations and the interactions between the fetus and the mother that characterize this period introduce special considerations for their treatment. The proper management of such disorders and the maintenance of euthyroidism during gestation are vital for avoiding adverse outcomes for both the mother and the fetus.

Research on thyroid pathophysiology during pregnancy has provided valuable insights in many of the mechanisms responsible for thyroid disturbances throughout this period and postpartum. However, controversies still exist and a consensus on screening for thyroid disorders during pregnancy has not been reached. Future research is expected to shed light in these matters and aid our attempts to ensure optimal conditions for both the mother and the fetus during the fascinating period of gestation as well as in later life.

4. ABSTRAKT ČESKY

Fyziologie štítné žlázy souvisí s mnoha biochemickými procesy lidského těla a vykazuje poměrně široké spektrum poruch. Hormony štítné žlázy jsou produkovány vazbou jodu ve štítné žláze. Tento proces je regulován jednak intrathyreoidálně enzymovými procesy, jednak extrathyreoidálně prostřednictvím osy hypothalamus-hypofýza-štítná žláza (HPTA, **H**ypothalamus-**P**iruitary-**T**hyroid-**A**xis). Dostupnost hormonů štítné žlázy a jejich účinky v periferních tkáních jsou regulovány speciálními enzymy – dejodinasami.

Těhotenství je stav výjimečných fyziologických změn pro matku i plod. Interakce mezi matkou a plodem jsou zprostředkovány placentou. Hormony štítné žlázy jsou životně důležité pro fyziologické procesy matky během těhotenství a mají vliv i na vývoj plodu. Zvýšená potřeba thyreoidálních hormonů během těhotenství klade zvýšené nároky na štítnou žlázu a příjem jodu. Neschopnost štítné žlázy adaptovat se na tento stav se projevuje zvýšeným výskytem komplikací, jako jsou preeklampsie, předčasný porod nebo dokonce smrt plodu nebo kretenismus.

K takovým stavům dochází také v případech preexistujících nebo získaných chorob štítné žlázy. Gravesova nemoc a Hashimotova nemoc jsou nejběžnější příčinou hormonální nerovnováhy během těhotenství. Immunitní změny a interakce mezi plodem a matkou, které jsou charakteristické pro toto období, vyžadují, aby jejich léčbě byla věnována speciální pozornost. Správné zvládání takových poruch a udržování fyziologických hladin hormonů štítné žlázy během těhotenství jsou nezbytné, nemá-li dojít k nežádoucímu poškození matky i plodu.

Výzkum patologického fungování štítné žlázy v průběhu těhotenství vedl k významnému objasnění mechanismů zodpovědných za thyreoidální poruchy během těhotenství a po porodu. Avšak stále existují nedořešené problémy a nebyl přijat konečný názor na sledování těchto poruch během těhotenství. Očekává se, že další výzkum osvětlí tyto záležitosti a napomůže našim snahám zajistit optimální podmínky jak pro matku, tak plod během nádherného období těhotenství i v pozdějším životě.

5. INTRODUCTION

Pregnancy is a state in which two organisms, the mother and the fetus, share common pathophysiological pathways, nutrients, and inevitably in certain cases, common pathology. This remarkable interplay of biological reactions is characterized by continuous variations in the physiology of both the maternal and the fetal organisms. As expected, diseases that affect the "host" mother will often have consequences for the fetus as well; pathology of the thyroid gland is no exception to the latter argument.

Thyroid disorders are common in the general population and especially in women. Therefore, a large number of women are affected by thyroid disorders during gestation causing either hyperthyroidism or hypothyroidism that can sometimes course undetected through gestation. Disorders such as Grave's disease, autoimmune hypothyroidism and postpartum thyroiditis pose difficulties in the management of pregnant women and require caution in order to ensure a healthy environment for both the growing fetus and the mother. Thyroid disease can affect gestation and its outcome in various ways: it can alter the ability to conceive prior gestation, it can raise the risk of adverse outcomes such as miscarriage as well as to increase the risk of obstetric complications such as placental abruption or preeclampsia. Thyroid pathology, left untreated, can adversely affect the normal development of the fetus leading to abnormalities as serious as the currently rare in developed countries, cretinism.

The major challenge in ensuring euthyroidism during gestation lies in the complexity of thyroid hormone physiology during this period. Thyroid hormones are produced according to the regulation of the hypothalamic-pituitary-thyroid axis and iodine intake. The fetus does not concentrate iodine before 10-12 weeks of gestation and the production of thyroidal hormones initiates at approximately 20 weeks of gestation (Leung, Millar et al. 1993). Prior to this period the fetus relies on the maternal production of thyroid hormones. The latter fact and the alterations in maternal estrogen levels as well as in the levels of human chorionic gonadotropin (hCG) are only some of the principal events leading to an increase in the demands of maternal thyroid hormone production during this period. Inadequate iodine intake or inability of the thyroid gland to cope to the high demands such as in the case of subclinical hypothyroidism lead to insufficient thyroid hormone levels for both the mother and the fetus. Depending on the stage of gestation such inadequacies can lead

to adverse events such as miscarriage, premature birth or even cretinism for the fetus. Studies have shown that timely intervention in order to promote euthyroid function is critical to ensure that the gestation period and its outcome are favorable for both the mother and the fetus.

However, most of the available tests have proven to be inefficient in terms of accuracy and reproducibility to assess thyroid function during pregnancy. Moreover, studies have failed to adequately present the necessity of screening for thyroid function during gestation and to provide a recommended optimal period for this procedure. Therefore a consensus on screening for thyroid pathology has yet to be achieved, although most of the major medical societies concerned by the issue offer very helpful guidelines. The same controversy exists on the recommended treatment regiments and the dosages prescribed during pregnancy for thyroid disorders, since some of which may raise the risk for adverse reactions for both the mother and the fetus.

In this diploma thesis, I will attempt to elucidate thyroid pathophysiology during pregnancy and its relation to the course and outcome of gestation for both the mother and the fetus. To accomplish this, I will begin by presenting current knowledge on maternal and fetal thyroid physiology and on their interactions. In this context I will present the most common disorders affecting the mother and the fetus and their impact on the outcome of gestation, as well as current concepts concerning their management. Finally, I will refer to the still existing on this issue since such controversies will set the goals for future research in the field.

6. THYROID PHSYIOLOGY: THE PRODUCTION, BIOAVAILABILITY AND EFFECTS OF THYROID HORMONES

6.1. Thyroid Synthesis of Tetraidothyronine and Triidothyronine

Tetraidothyronine (or thyroxine T4) and Triidothyronine (T3) constitute the hormones through which the thyroid gland exerts its effects on the homeostasis and physiology of the human body. T3 and T4 are included in the family of proteins called amines and their production is based on the biochemical modulation of the aromatic amino acid Tyrosine (Tyr) or 4-hydroxyphenylalanine. The biochemical structure of the hormones is evident in **Figure 1.**

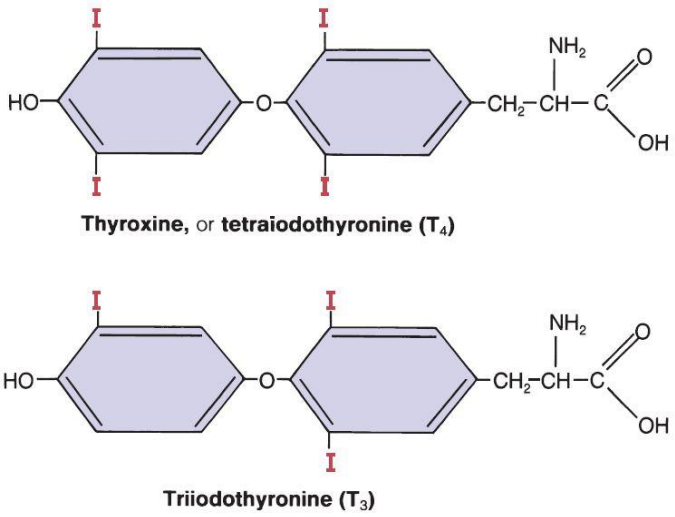

Figure 1. Figure illustrating the biochemical structure of Triiodothyronine (T3) and tetraiodothyronine (T4). (Image obtained from the book: "Human physiology, twelfth edition" by Stuart Ira Fox, Chapter 11, p.315.) (Fox 2011).

As already stated, T3 and T4 are produced in the thyroid gland through the sequestration of complex biochemical machinery. The production of thyroid hormones is regulated by a complex feedback mechanism that involves both extrathyroid and intrathyroid pathways and the function of the pituitary gland and the hypothalamus, as will be explained further. However, prior to the discussion on the regulation of the thyroid hormones and its alterations however, it is considered imperative to illustrate the metabolic pathways that govern their production by the thyroid gland.

The synthesis of T3 and T4 takes place in thyroid's dominant structure, the thyroid follicles, which consist of a clear proteinaceous colloid filled lumen enclosed by an assortment of follicular cells. Each follicular cell has a "bipolar" orientation, with its apical membrane bordering with the colloid substance and the basolateral membrane bordering with the extrafollicular space that is accessible to the blood stream. Thyroid hormones are synthesized in the follicular cells through a complex procedure that results in the iodization of tyrosine and are then released into the bloodstream (Mansourian 2011).

As illustrated in **Figure 2**, iodine, the basic element for thyroid hormone production, is actively transported into the thyroid follicular cells through the action of a plasma membrane called the sodium-iodine-symporter (NIS). NIS creates an iodine concentration 40 times higher than the circulating levels, providing an abundance of this substrate for hormone production that depends on the availability of iodine in the bloodstream as will be analyzed further. Iodine is subsequently oxidized to iodide by an enzyme called thyroid peroxidase (TPO) in the presence of H_2O_2. Oxidized iodine is then transported into the colloid-filled follicular lumen through the action of an iodine-chlorine transporter called pendrin. The latter events constitute a mechanism called "*iodine trapping*" that is fundamental to the exploitation of the available iodine for hormone production.

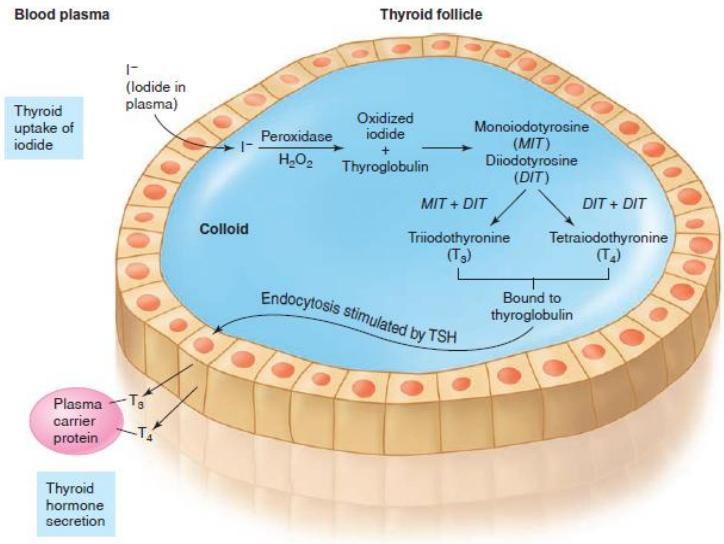

Figure 2. Image illustrating the mechanisms of Triiodothyronine (T3) and Tetraiodothyronine (T4) production by thyroid cells, and the utilization of iodine. (Image obtained from the book: "Human physiology, twelfth edition" by Stuart Ira Fox, Chapter 11, p.338) (Fox 2011)**.**

Within the colloid, iodine is coupled with tyrosine residues on the molecule of thyroglobulin (TG), a glycoprotein synthesized by the follicular cells. Thyroglobulin is a 660 kDa macropriotein containing approximately 5000 aminoacids and 115 tyrosine residues, and serves both as a mean of tyrosine iodization and hormone production and as a storage protein for thyroid hormones in the colloid. The iodination of tyrosine is mediated by the enzyme TPO and the resulting substances are monoiodotyrosine (MIT) and diiodotyrosine (DIT) when one iodine molecule or two iodine molecules are transferred to tyrosine respectively. TPO mediates another process through which MIT and DIT are combined to form T3 and two molecules of DIT are combined to form T4 (de Vijlder, Ris-Stalpers et al. 1997, Kopp 2000). From the follicular lumen, small amounts of colloid are absorbed from the apical surface of the follicular cells and into membrane vesicles through the mechanism of pinocytosis (Rousset, Mornex 1991). TG is then incorporated into phagolysosomes and undergoes proteolytic digestion by lysosomal enzymes and the end products of this process are MIT, DIT, T3 and T4. Subsequently, T3 and T4 are released into the blood circulation while TG, DIT and MIT can be re-cycled and utilized in further hormone production cycles (Mansourian 2011). Thyroid hormones circulate into the bloodstream in different ratios, with serum T4 levels being 40-fold higher than T3 (Glinoer 2004).

6.2. Hypothalamic-Pituitary-Axis and the role of TSH

Thyroid hormone production is governed by complex regulatory mechanisms aiming to preserve homeostasis by preventing excess hormone production that leads to hyperthyroidism or thyroid hormone deficiency, which is known with the clinical term “hypothyroidism”. This complex regulation is mediated through the hypothalamic-pituitary-thyroid axis but also through intracellular feedback mechanisms in the thyroid gland. The thyroid production signal of the hypothalamus is mediated through the Thyrotropin-Releasing-Hormone (TRH), a peptide hormone released by the paraventricular nucleus of the hypothalamus, transported to the median eminence and from there to the anterior pituitary through the portal capillary plexus. TRH mainly serves as a stimulator for the release of Thyroid-Stimulating-Hormone (TSH) from the pituitary cells. Its effect is mediated through the TRH receptors on a subgroup of TSH producing cells called pituitary thyrotrope cells. TRH receptors belong to the seven-transmembrane spanning receptor family and are coupled to Gq11. This regulatory mechanism is known as the Hypothalamic-Pituitary-Axis (HPTA) and is presented in **Figure 3.**

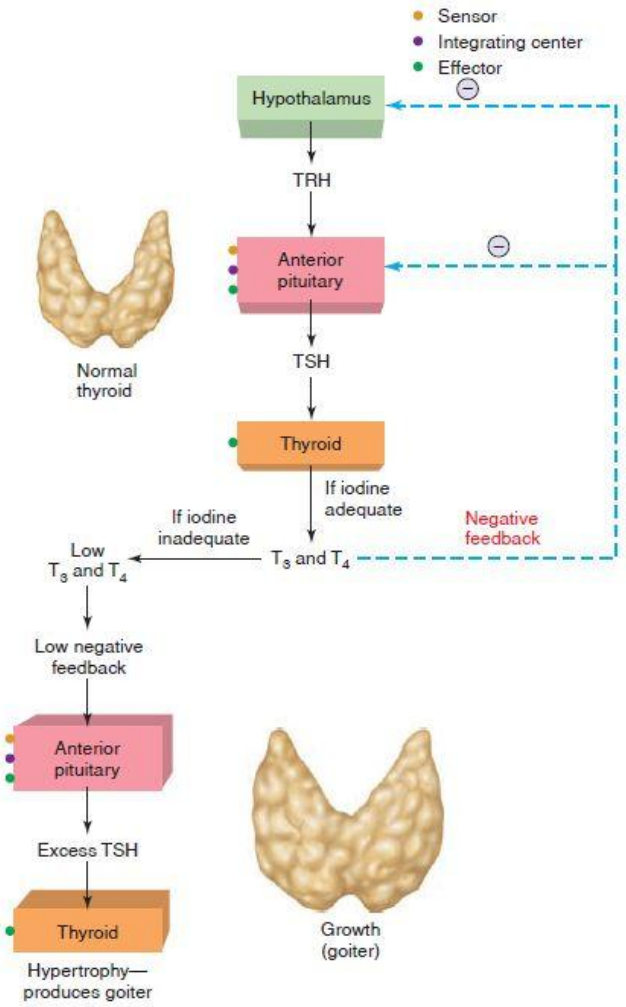

Figure 3. Figure representing the Hypothalamic-Pituitary-Thyroid axis with additional information on the role of iodine deficiency in goiter formation, as will be discussed later. From the book: "Human physiology, twelfth edition" by Stuart Ira Fox, Chapter 11, p.339) (Fox 2011).

The pituitary hormone TSH, also known as thyrotropin, is a 28 kDa glycoprotein consisting of two subunits, the α-subunit and the β-subunit. While the 118 amino-acid β-subunit is specific to TSH and determines its function, the α-subunit consisting of a sequence of 92 amino-acids is common between TSH and other hormones such as the chorionic gonadotropin (hCG), follicle stimulating hormone and luteinizing hormone (Yen 2001). This similarity is the basis of a "spill-over effect" in the action of hCG during pregnancy, that is postulated to exert thyroid stimulation through the mimicry of TSH action (Fondell, Roy et al. 1993). The production of TRH and TSH is subject to a negative-feedback mechanism which is based on the levels of

T4/T3 and that, in the case of TSH probably involves the intrapituitary conversion of T4 to T3 by the enzyme type II deiodinase (Yen 2001).

TSH facilitates its effects through its binding on specific, seven transmembrane-spanning TSH receptors (TSHr) coupled to Gs that are present on the follicular cell membrane. Receptors of similar structure are responsible for mediating the effects of TRH and hCG as well. TSH acts through a cascade of alterations in the expression and function of enzymes and transporters involved in thyroid hormone production. It stimulates the expression of genes coding the NIS symporter, thyroglobulin (TG) and thyroid peroxidase (TPO) through cyclic adenosine monophosphate (c-AMP) (Alquier, Ruf et al. 1989, Fayadat, Niccoli-Sire et al. 1998, De Deken, Wang et al. 2000). Furthermore, it increases the activity of NIS promoting the uptake of iodine, and the iodination of tyrosine residues on TG (Yen 2001). It is worth mentioning, that the circulation of autoantibodies against TSH receptors is responsible for the overt thyroid stimulation observed in Graves' disease, while mutations on TSH receptors can lead to the autonomous stimulation observed in Plummer's adenoma and toxic adenoma (Perros 2005, Gaberscek, Zaletel 2011).

6.3. Thyroid Self-Regulation and the role of Iodine

In addition to the extrathyroidal regulation of thyroid hormone production, a self-regulatory mechanism exists in the gland itself that in special circumstances halts the production of THs through a mechanism that involves iodine. Wolff and Chaikoff were the first to describe this role of iodine in 1948, which is since known as the Wolff-Chaikoffeffect (Wolff-Chaikoff, 1948). According to the latter, when iodine is in excess concentrations in the thyroid, the production of THs is halted; various mechanisms have been proposed to explain this phenomenon with the most widely accepted being the alteration of H_2O_2 production by TPO through its inhibition by the extra iodine and the interference of iodinated lipid residues with the enzymatic machinery required for H_2O_2 production and Tg iodination (Marino 2000, De vijlder1992).

6.4. Thyroid Hormone Transport, Storage and Peripheral Action

The amount of free circulating thyroid hormones represents only a small fraction of their total amount. The latter is due to the fact that T3 and T4 are bound to

transport, proteins, which are: thyroxine-binding globulin (TBG) (approximately 70%), albumin (15-20%) and thransthyretin (the previously called prealbumin or TBPA) (10-15%) (Karapanou, Papadimitriou 2011). In steady state, equilibrium exists between the total amount of TH and the amount of free circulating T3 (approximately 0.5%) and T4 (approximately 0.04%) (Mansourian 2011). It is worth mentioning that only the unbound forms of THs are biologically active and that false assumptions can be made when measurements of THs are based on measuring their total amount instead of their free forms or of ratios indicative of the actual thyroid state such as T3/T4 and T4/TBG (Glinoer 2004). Beside its low concentrations compared to the other transport proteins, TBG represents the major "extrathyroidal pool" and alterations to its affinity or abundance can lead to alterations in the percentage of the free form (short term) and on the total amount (long term) of T4. The explanation behind this role of TBG lies in the high affinity of this 54kDa liver produced protein, for thyroxin (T4) (Murata, Magner et al. 1986, Visser, Friesema et al. 2008).

6.5. Thyroid Hormone Metabolism and Peripheral Action

The production of thyroid hormones is not limited to the processes taking place in the thyroid gland. T3 also derives from 5'-deiodination of T4 and as a matter of fact, this process accounts for the majority of the circulating T3. The process of deiodination is mediated by enzymes called deiodinases, which through this process can lead to the conversion of THs but also to their inhibition and degradation (Kohrle 2000). Deiodinases are membrane proteins with their active sites located in the ,cytoplasm , are found in peripheral tissues and play a crucial role in the peripheral action of THs as well as on their metabolism (Bianco, Kim 2006). Namely, type I deiodinase converts the majority of T4 to T3 and exists in the kidneys, the liver and other peripheral tissues. The conversion of T4 to T3 for intracellular use is mediated through Type II deiodinase which is found in brown adipose tissue, the brain and the pituitary gland. Type III deiodinase is of particular importance for the physiology of thyroid hormones during pregnancy since it is found in the placenta, brain and skin and is responsible for the inactivation of both T3 and T4 through the deiodination of their inner ring (Yen 2001). TH metabolism also shares similarities to the metabolism of bile salts; i.e the deiodination T3 and rT3 also takes place in the liver where they are further conjugated, excreted in the bile and partly reabsorbed through the enterohepatic circulation (Engler, Burger 1984).

Despite their characterization as amines, THs exert their effect to the body through molecular procedures similar to steroid hormones. This characteristic of their action can explain their major importance in regulating the metabolism and development since the fetal life. Unbound THs enter the cells of peripheral tissues through the mediation of hormone receptors/transporters on the cell membranes such as the monocarboxylate transporter 8 (MCT8), anion transporter polypeptides (OATP) L-type amino acid transporters (LAT1 and LAT2) and other similar molecules (Visser, Friesema et al. 2008, Visser, Friesema et al. 2011). Intracellularly, they translocate into the nucleus and bind to specific receptors, called thyroid hormone receptors (TR); TRs are associated with chromatin and are bound to thyroid responsive elements (TREs) through which the transcription of target genes and subsequently the synthesis of specific proteins is enhanced or inhibited. Thus, TRs have been characterized as "ligand-regulatable transcription factors" (Samuels, Forman et al. 1989, Yen 2001). An overview of this process is illustrated in **Figure 4**.

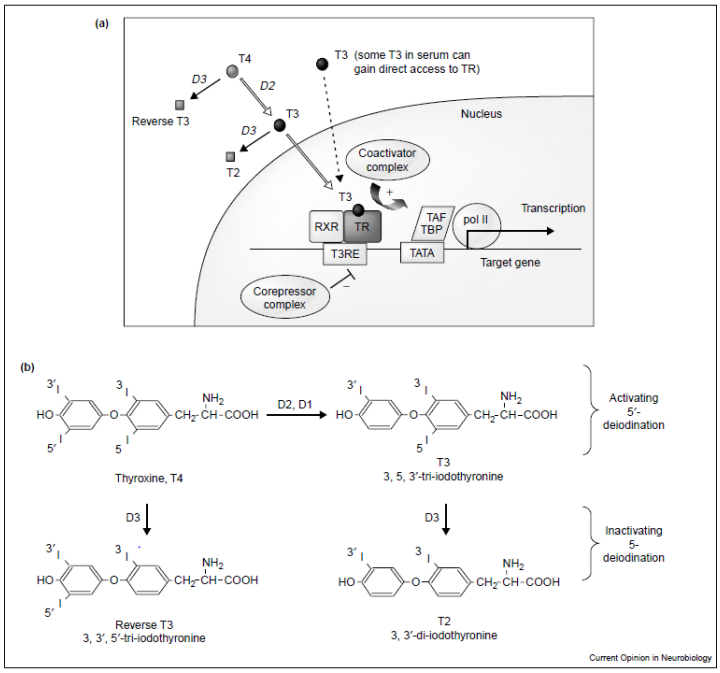

Figure 4. Diagram illustrating the activating-deactivating role of deiodinase enzymes in peripheral tissues and the entrance of the active forms of thyroid hormones in the cell nuclei to subsequently bind to thyroid responsive elements and exert their metabolic actions.

The effects of THs are also mediated by non-nuclear mechanisms, such as the interaction with membrane receptors coupled to G proteins, the interaction with

protein kinases and the alteration of intracellular calcium concentrations (Yen 2001, Warner, Mittag 2012).

Through “genomic and non-genomic” interactions, the different types of “iodothyronines” regulate the heart rate, metabolism and development through a variety of processes that exceed the purpose of the current review. Further discussion of specific mechanisms such as fetus development and heart rate will be discussed further in the respective chapters.

7. THYROID FUNCTION DURING PREGNANCY

Thyroid hormones undergo some significant alterations during the gestation period, due to the adaptation of the thyroid gland to the increased needs of the pregnant state. As shown in **Table 1**, experimental studies suggest an increase in total T4 in the bloodstream during the first trimester, however T4 levels seem to decrease to the lower normal values during the progression of gestation (Berghout, Wiersinga 1998, Sapin, d'Herbomez 2003).

Table 1. Thyroid physiology and autoimmunity in pregnancy and after delivery.

Parameter	First trimester	Second trimester	Third trimester	After delivery
hCG	↑↑	↘ →	↘	↓↓
TSH	↘↓	↗	↗	↘
fT_4	↗↑	↘	↘↓	↗
fT_3	↗↑	↘	↘↓	↗
Treg	↑	↑↑	↘	↓↓
TAb	↘	↓	↓↓	↑↑

→: No change; ↘: Slight decrease; ↓: Decrease; ↓↓: Marked decrease; ↗: Slight increase; ↑: Increase; ↑↑: Marked increase when compared with the previous period.
fT_3: Free triiodothyronine; fT_4: Free thyroxine; hCG: Human chorionic gonadotropin; TAb: Thyroid autoantibodies; TSH: Thyroid stimulating hormone.
Data taken from [2,3,14,18–24,49,52,55].

Table 1. Table summarizing our current knowledge on thyroid hormone alterations and the relative fluctuations of hormones and antibodies associated with thyroid function. Table obtained from a study by Gaberscek and co-workers, on thyroid function and autoimmunity during pregnancy (Gaberscek, Zaletel 2011).

Moreover, the free forms of both T3 and T4 have been shown to decrease during pregnancy, with their levels resting below their respective values in nonpregnant women as well as below reference values in many occasions. This decrease seems to reverse during the postpartum period, given that iodine intake is sufficient (Yu, Wang et al. 2010). Such a decrease in fT4 could be intuitively explained by a raise in iodine requirements during this period, accompanied by an insufficiently increased intake to compensate and leading to a state of iodine deficiency during pregnancy. However, as Fister P. et al suggest in their study, and as

suggested in similar studies, iodine status does not seem sufficient to explain the fluctuations in fT4 and fT3. Fister P. et al have observed a decrease in fT3 and fT4 during the third trimester that was not correlated with iodine urine concentrations and did not imply a causal relationship with iodine status (Laurberg, Andersen et al. 2007, Fister, Gaberscek et al. 2011). It is important to clarify, that while iodine intake is not sufficient to explain TH alterations in all pregnant women, it is however a major contributor to thyroid disease during pregnancy with major implications for the fetus, and therefore this topic will be analyzed further in this study. In respect to the latter, it is evident that in order to delineate each component of thyroid physiology adaptations, other factors such as iodine intake must be kept constant. Therefore the discussion on such findings is important to be based on studies conducted on women without iodine deficiency, except in cases that the latter is the issue investigated. An increase in fT3 has further complicated the aforementioned observations, leading to considerations of nonthyroidal illness attributing to these effects (Berghout, Wiersinga 1998).

7.1. TSH Levels and Correlation with Thyroid Adaptation

Another proposed explanation for the observed variations of THs, is that the rise of TBG concentrations and therefore of its binding capacity even without changes in its binding affinity, could lead to binding of much of the free forms of the hormones and therefore to a fall in their serum levels. Such an event would be expected to cause a shift of T4 to its bound form causing a fall in fT4 levels that could be transient should the thyroid gland be able to compensate for this alteration. Therefore the mechanism proposed by this speculation would also predict a rise in thyroid activity marked by alterations on TSH levels, thyroid volume or other markers of thyroid activity. Indeed, it seems that TBG fluctuations exhibit a temporal correlation with changes in fT4, albeit not absolute, as indicated by Gaberscek et al (Gaberscek, Zaletel 2011). Moreover, as evident in **Table 1,** fT4 changes are accompanied by alterations in TSH and its behavior during pregnancy has been extensively researched due to its role as a major contributor to thyroid disease in general and as a marker of thyroid function due to its close association to thyroid hormone production by the gland through the hypothalamic-pituitary-thyroid axis (HPTA) regulation. An assumption could be made, before testing the role of TSH in TH alterations, that the HPTA is not altered in any way during pregnancy. Early studies dating before 1980 suggest that the latter is true mainly by testing the pituitary and thyroid response after TRH administration; their analysis conducted by Burrow et al. in a 1993 review concludes

that the HTPA remains unaffected during pregnancy (Burrow et al, 1993). Although some caution would be required considering the date of the studies, their results can be considered as valid, according to Glinoerand coworkers (Glinoer 1997).

Concerning TSH levels, observations suggest an increase of TSH during the gestation period, followed by a decrease after delivery (Shields, Ward 2001) and that in a period of 4-months postpartum and 1 year postpartum TSH levels appear to be lower than in the third trimester of pregnancy (Soldin, Tractenberg et al. 2004). However, the latter conclusions are refuted by more recent studies, suggesting that this could be an effect of iodine deficiency of the subjects due to the location of the studies (in European countries with iodine restriction) (Glinoer 1997). It seems most probable that TSH remains unchanged during most of the second half of gestation. An exception to the latter is the transient fall of TSH occurring during the first trimester, observed in approximately 10% of women having lower than normal values and 10% having suppressed TSH values (Shields, Ward 2001).

The rise in fT4 and the decrease in TSH during the first trimester imply a thyroid stimulatory factor other than TSH according to the normal HPTA axis regulation, since the normal pathway of thyroid compensation due to fT4 lowering would include a rise in TSH so this is ruled out by such evidence. Such factor would cause an increase in fT4 accompanied by a negative-feedback mediated decrease of TSH and would certainly be a factor arising or increasing during pregnancy; as studies suggest, such a role can be attributed to the hormone named human chorionic gonadotropin also known as hCG.

7.2. The Role of hCG in Thyroid Physiology during Gestation

The failure of the initial attempts to prove a reciprocal relation between TSH and hCG, lead to the assumption that human chorionic thyrotropin (hCT), a placental derivative with thyroid stimulating action similar to TSH, could be responsible for the observations (Braunstein, Hershman 1976). However later studies refuted the latter and through experiments conducted in molar and twin pregnancies and choriocarcinoma cases suggested more efficiently that hCG or its biological variants could be responsible for TSH blunting and T4 alterations (Mann, Hoermann 1993). This notion was proved by later studies, such as the study conducted by Pekonen et al revealing a negative correlation of TSH to hCG (Pekonen, Alfthan et al. 1988). These results are more profoundly reported by Glinoer et al who in their prospective study

managed to provide evidence of a "mirror" image of hCG and TSH during the first trimester, accompanied by a rise in fT4 (Glinoer 1997). Thus, hCG is indeed an important regulator of thyroid function during pregnancy. The homology between the β-subunits and the receptors of hCG and TSH, seem to explain these observations (**Figure 5)**. A study by Glinoer et al. clearly demonstrates this association, by showing a blunting of TSH that decreases accordingly to the "amplitude and duration of the hCG peak", an effect that seems to be higher in twin pregnancies (that are characterized by higher levels of hCG and therefore higher thyrotrophic effect) leading to higher fT4 and lower TSH in such cases. It should also be noted that this effect of hCG leads to hyperthyroidism in certain cases, the prediction of which is the aim of research in the field (Grun, Meuris et al. 1997).

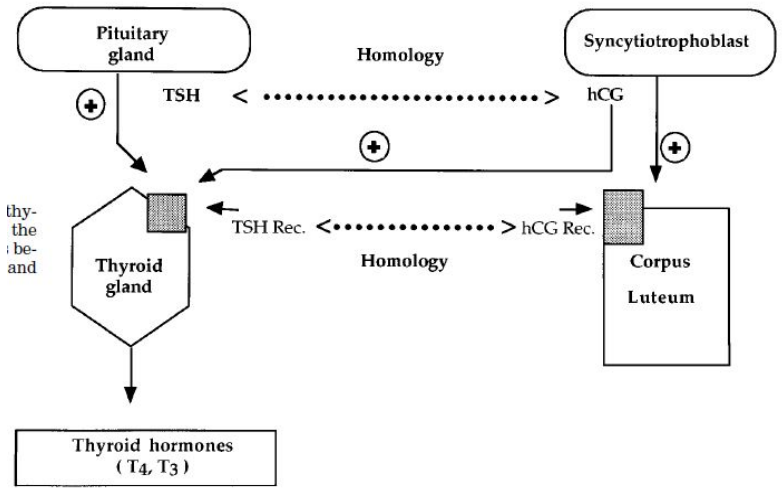

Figure 5. Figure by Glinoer et al describing the homology between TSH and hCG receptors and its consequences on thyroid stimulation through a "spill-over" activity of hCG, responsible for many of the observations on thyroid function during pregnancy (Glinoer 1997),.

According to these findings, the thyroidal economy during pregnancy seems to be regulated by a two-fold and contradicting action of hCG during the first trimester. Firstly, hCG leads to estrogen production that subsequently promote an increase in TBG concentrations and a decrease in fT4 that according to the expected HPTA compensation should drive an increase in TSH and an expected compensatory increase in fT4. However, at the same time, due to its thyrotrophic action hCG leads to a blunting of TSH and consequently promotes an increase in fT4 through an antagonistic mechanism for thyroid stimulation with TSH. Considering the variability of iodine intake between different populations and subjects and the variability of different

measurement methods, the ambiguous findings of early studies in the subject become perfectly clear. As a matter of fact, one would have to admit that the associations delineated through such studies in the past deserve much credit (Glinoer 1997). The effects exerted by hCG to the thyroidal economy are amplified or complicated by iodine deficiency, thus iodine intake has a major role in achieving euthyroidism during pregnancy and iodine deficiency has important implications for both the mother and the fetus.

7.3. Iodine Efficiency and its effects on Thyroid Economy in Pregnancy

The effects of iodine deficiency range from endemic hypothyroidism and goiter to the extreme for modernized societies event of endemic cretinism. The World Health Organization has developed certain guidelines for the recommended daily intake of iodine and suggests programs of increasing this through iodination of salt and other policies, although these are note employed universally (Delange, Burgi 1989, Delange 1994). In many European countries such as Belgium, the iodine intake is marginally low to a level of 50-100 μg/day. Iodine deficiency is more evident in Central Africa and Asia, with iodine intake levels as low as 25μg/day. Selenium deficiency and the presence of goitrogens in the diet of certain populations further increases the effects of iodine deficiency and leads to endemic goiter prevalence as high as 60-70% of adults (Ermans 1994, World Health Organization).

As illustrated in **Figure 6**, pregnancy is a state marked by considerable changes in iodine needs leading to iodine deficiency if adequate adjustments in iodine intake are not employed.

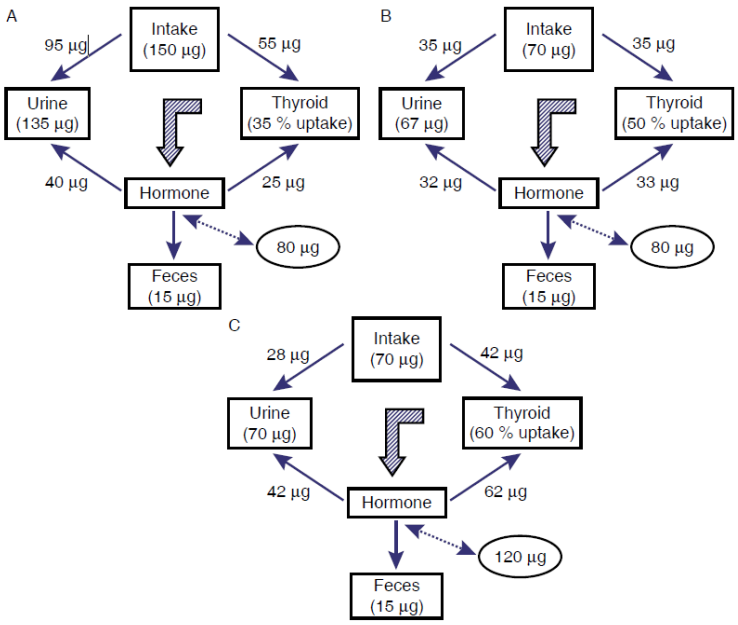

Figure 6. Schematic representation of the kinetics of iodide in healthy non pregnant and pregnant adults derived by a paper of Glinoer et al.

Panel (A): non-pregnant adult with adequate iodine intake (150 mg/day). Panel (B): non-pregnant adult with restricted iodine intake (70 mg/day). Panel (C): the latter condition is compared with an identically restricted level of iodine intake (70 mg/day) in a pregnant woman. Daily TH production was set at 80 mg of iodide/day (non - pregnant) and increased by 1.5-fold to 120 mg/day during pregnancy (Glinoer 2004).

The increase in iodine needs during pregnancy due to thyroid stimulation, has led to the suggestion that pregnancy could be a factor amplifying iodine deficiency and inducing thyroidal pathology and goiter formation (Glinoer, Lemone 1992). Measurements of thyroid function are therefore considered helpful in diagnosing hypothyroidism early and proceeding to iodine supplementation to prevent any complications. Iodine intake is usually estimated indirectly through measurements of iodine excretion in urine. Such measurements can provide evidence of iodine deficiency, however they suffer from the fact that they offer estimates of iodine intake extending to a period of days prior to the test instead of providing evidence of the more important long-term iodine status (Glinoer 1997).

Therefore, other parameters of thyroid function have been suggested for defining iodine deficiency during pregnancy, such as the relative hypothyroxinemia, the T3/T4 molar ratio and TSH and TG levels in the serum. Relative hypothiroxinemia is observed in women who, due to restricted iodine intake and therefore iodine

deficiency during pregnancy, fail to exhibit the expected adaptation observed during the rise of TBG which is normally followed by a rise in total T4. Instead of the expected findings, TBG increase is accompanied by a less than estimated increase in total T4, with women that are in the lower tertiles of the population range having a high risk of remaining in the low range during late gestation. Moreover, this observation seems to be corrected through the administration of iodine supplements, proving a causal relationship of inadequate thyroid adaptation to high TH demands and iodine deficiency (Glinoer 1997).

Another important parameter is the T3/T4 molar ratio in the serum that in the setting of sufficient iodine intake is expected to remain stable during gestation due to differences in respective affinities of T4 and T3 with TBG (Fresco, Curti et al. 1982). This ratio has been shown to increase in cases of iodine deficiency and this has been attributed to a preferential T3 production by the thyroid owing to the limited intrathyroidal iodine pool. The same effect can be exerted by increased TSH stimulation of the thyroid but also reflects iodine and has been therefore suggested as a useful marker of iodine efficiency during pregnancy (Delange, Burgi 1989, Berghout, Endert et al. 1994, Glinoer 1997). The T3/T4 ratio has been used by researchers to show that the effects of iodine restriction during pregnancy persist for a long period postpartum (Glinoer, Lemone 1992, Glinoer 2004).

Another important indicator of thyroidal stimulation under certain conditions, utilized extensively in the past to assess thyroid function is TSH response as a part of the HPTA. As already mentioned, in periods of increased thyroidal needs, thyrotropin (TSH) levels elevate in order to stimulate increased production by the gland, as a compensatory mechanism. As could be expected, a similar reaction takes place in situations of increased needs such as gestation and is amplified by restrictions in resources such as iodine. Research observations report a rise in TSH levels after the first trimester that is continued until term. Such an increase in serum TSH can be moderate in territories with moderate iodine restriction such as Brussels with TSH levels of 0.75 mU/liter, to high in territories such as Zaire where iodine restriction is also severe with TSH values reaching 100 mU/liter in some women. The latter study also investigated iodine supplementation and revealed a positive effect of iodine supplementation with regiments such as 1ml of iodized oil in the second trimester, that was followed by a decrease of TSH to values below 20mU/liter at delivery (Thilly, Delange et al. 1978).

Finally, Thyroglobulin (TG) concentrations are also associated with iodine restriction and TSH elevation but also with goiter formation. Thyroglobulin is a potential marker of thyroid activity, since it is increased with thyroid hyperstimulation, goiter or injury of the thyroid (Spencer, Wang 1995), and is frequently increased during gestation (Glinoer 1997). In a study by De Escobar and coworkers an increase TG levels by approximately 60% accompanied a 100% increase in TSH levels and was also reduced after iodine supplementation (Spencer, Wang 1995, de Escobar, Obregon et al. 2004).

The aforementioned observations suggest that T3, T4, TSH, TBG and TG measurements could aid thyroid assessment during gestation as markers of thyroid adaptation and activity. A summary of the observations mentioned on thyroid alterations during pregnancy is provided in **Figure 7.**

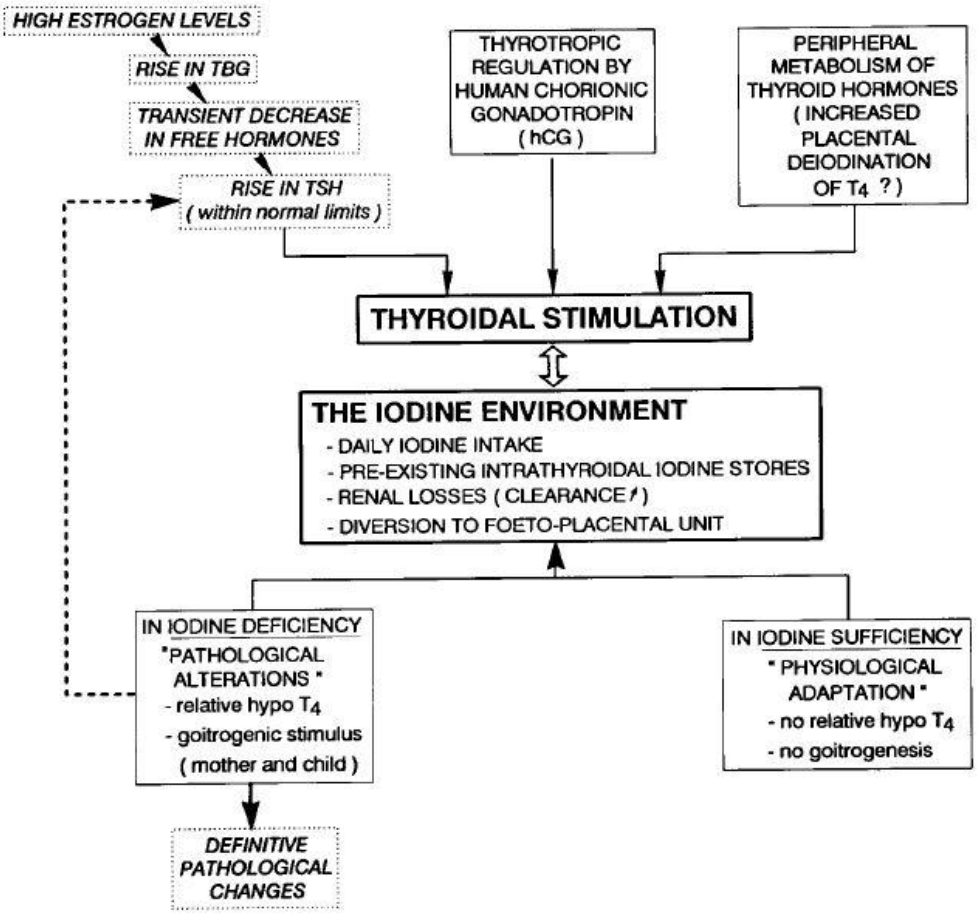

Figure 7. This figure summarizes the effects of pregnancy on thyroidal economy and thyroid adaptation, which in certain situations result in adverse effects. The role of iodine deficiency on thyroid stimulation is emphasized. Figure obtained from a review study by Glinoer and coworkers (Glinoer 1997).

8. FETAL THYROID HORMONES AND THE DEVELOPMENT OF THE FETAL BRAIN

Thyroid pathology during pregnancy and its consequences to the mother during the gestation period and postpartum, have been extensively investigated in pregnant women. However, the pregnant mother and the fetus are characterized by interactions governed by complex mechanisms and while in some aspects they can be regarded as separate organisms sharing common resources, in other aspects they present distinct functions and physiology with interesting consequences. The latter fact, and the importance of thyroid hormones for normal fetal development, set the fetal thyroid physiology as a central subject of research on thyroid effects in pregnancy. According to the known mechanisms underlying thyroid physiology in humans, it would be expected that many potential mechanisms could be underlying maternal and fetal thyroid function. It is possible therefore, that the maternal thyroid gland is the main contributor of thyroid hormones to the fetus; another possibility would be that the fetus produces its own hormones either intrathyroidally or extrathyroidally or even that it is based on the metabolism and processing of maternal hormones for its needs (Fisher 1997). The effects of thyroid hormones on fetal life are of particular interest both in their role in fetal development as well as in their potential toxicity on fetal tissues and their effects on fetal physiology in general and therefore their origin and regulation is of vital importance during gestation (de Escobar, Obregon et al. 2004).

8.1. Before the Onset of Fetal Thyroid Function

The investigation of thyroid hormone levels and their role in fetal development pose certain difficulties pertaining to our inability to obtain reliable measurements from the fetal serum and tissues due to ethical restrictions. Therefore, early research in the field, was solely based on the assessment of maternal thyroid function, on fetal and maternal complications due to thyroid abnormalities during pregnancy and on the outcomes of gestation concerning the development of the newborn. Such studies attempted to examine the association of maternal thyroid function and congenital hypothyroidism and cretinism especially in areas with iodine restriction and to evaluate the effects of treatment regiments in such cases. The classic studies in villages of the Himalayas by McCarrison et al during the first decades of the 20th century correlated maternal thyroid defects with thyroid abnormalities and cretinism in newborns (McCarrison R. 1917,de Escobar, Obregon et al. 2004). More valid

observations of fetal thyroid physiology were made in the 1980s with the use of radiolabeled iodothyronines to show that they are transferred from the maternal serum to the fetus and of Radioimmunoassays to measure small quantities of iodothyronines in fetal tissues.

However, it is the development of transvaginal ultrasound-guided puncture that enabled researchers to directly measure TH levels in embryonic cavities. Contempre B. and coworkersd etected and measured the levels of T3, rT3 and T4 in the coleomic and amniotic fluids during the first semester (Contempre, Jauniaux et al. 1993). THs were more abundant in the coleomic than in the amniotic fluid; T4 levels were ten-fold higher than T3 levels and correlated to maternal circulating concentrations albeit much lower than maternal values. Reverse T3 (rT3) was higher than T4 suggesting that the placenta barrier system was a significant regulator of the bioavailability of THs to the fetal circulation. Further studies revealed that despite the low concentrations of bound forms of THs, fT4 was found in levels similar to those of the maternal serum, implying a possible biological activity to the fetal tissues (Calvo, Obregon et al. 1990). The anatomical relations of the fetus, the placenta, the amniotic and the coleomic cavity are delineated in **figure 8**.

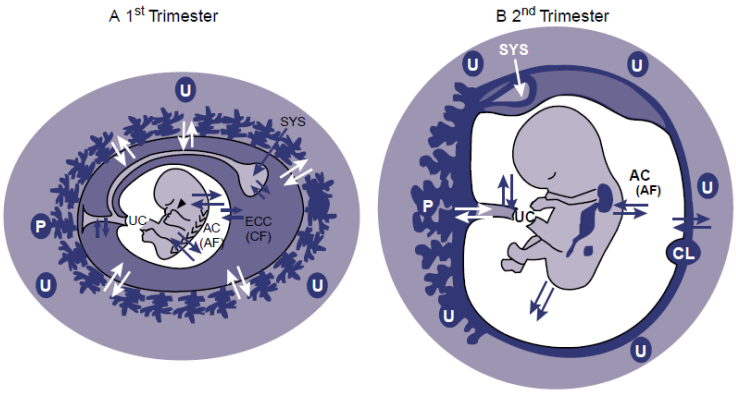

Figure 8. Figure illustrating the major cavities of the maternal-fetal unit during the 1st (A) and 2nd (B) trimesters of gestation. (A). The amniotic cavity (AC) is the cavity which directly surrounds the fetus and contains the amniotic fluid while the exocoleomic cavity (ECC) contains the coleomic fluid (CF) and the secondary yolk sac (SYS) and separates the amniotic sac and the placenta. (B). At the end of the 1st trimester the SYS and part of the placenta degenerate and the AC increases and the maternal-fetal exchange pathways are altered. Thyroid hormones as well as nutrients are directly transferred to the fetal circulation via the placenta. U, uterus; UC, umbilical cord; CL, chorion leave (membranes in development). Image derived from a review by De Escobar and coworkers (de Escobar, Obregon et al. 2004).

Subsequently, it was proved that fT4 levels depend on the maternal T4 and fT4 reaching the fetal circulation by "escaping" the placental barrier as well as on the binding capacity of proteins in fetal fluids that is considered to be stably low (Calvo, Obregon et al. 1990, de Escobar, Obregon et al. 2004).

Later studies revealed that the levels of T3 in the fetal brain reach levels equal to 34% of adult values (Ferreiro, Bernal et al. 1988), and even close to 100% of the adult levels at 13-20 weeks postmenstrual age (PMA) (Calvo, Obregon et al. 1990). This abundance of T3, contradicted the evidence on circulating T3 in the fetal serum, and prompted to the investigation of its sequestration by fetal tissues for the regulation of development and differentiation. Bernal and Pekonen investigated the concentration and activity of TRs in the fetal brain and revealed a ten-fold increase by 18 weeks (Contempre, Jauniaux et al. 1993) and their occupation by T3 reached 25-30% throughout the study (Ferreiro, Bernal et al. 1988). Their findings suggested that T3 had a major role on fetal brain development, elaborated as early as the first trimester of gestation.

The contradicting levels of T3 in the fetal brain were explained by subsequent studies that investigated the role of deiodinases in brain differentiation. To explain this observation, the activities of D1, D2 and D3 were studied in different areas of the brain in fetuses of 13-20 weeks PMA and in premature infants deceased around 24-42 weeks PMA. The levels of T3 corresponded to the activities of D3 and D2; the T3-producing D2 was detected in high concentrations in the cerebral cortex at the same period that T3 showed an increase in concentration and at the same time the inactivating D3 was low, while the opposite effects were observed in the cerebellum and other tissues with low values of T3. Thus, it seems that the bioavailability of T3 in the human brain during its development shows a temporal and spatial sequence closely correlated with cerebral differentiation, and this phenomenon is at least partly regulated by the activity of deiodinase enzymes (Karmarkar, Prabarkaran et al. 1993).

8.2. After the Onset of Fetal Thyroid Function

The onset of fetal thyroid function (FTF) at around 18-20 weeks PMA, could be expected to introduce alterations in the values of thyroid hormones in the fetal serum. However, studies have revealed that T3 and fT3 remain in low concentrations in the fetal serum and that T4 and fT4 continue to rise during the gestation period until term and approximate the adult values during the third trimester (Fisher 1997). An

interesting result has been revealed however, concerning the concentrations of TSH. Thorpe-Beeston et al observed that TSH concentrations were higher than maternal levels and were not a part of a negative feedback regulatory mechanism as observed in adults, but instead were positively correlated with fT4 values, an observation that opposed current knowledge on thyroid regulation (Calvo, Obregon et al. 1990, Thorpe-Beeston JG, 1991).

Subsequent studies revealed that TSH is possibly related to extra-thyroidal effects independent of the normal cAMP-mediated regulation, a finding that could possibly explain the lack of feedback control by fT4 concentrations (Saunier, Pierre et al. 1993). Moreover, it has been shown that despite thyroid activity in the fetus, the increasing levels of fT4 T4 are based on a combination of hormone production by the thyroid and an increasing amount of maternal hormones escaping the placental barrier (Koopdonk-Kool, de Vijlder et al. 1996, Santini, Chiovato et al. 1999). This persistent dependence on maternal THs even after the initiation of thyroid activity sets the fetus in a disadvantaged position in case of maternal hypothyroidism. The activity of deiodinases however has been found to compensate for any inefficiency to a certain degree, by providing higher amounts of T3 to the fetal brain through the activity of D2 and by deactivating less THs through the regulation of D3 activity (Calvo, Obregon et al. 1990).

A summarizing figure (**Figure 9),** provides an overview of the observations on the maternal and fetal thyroid hormone alterations during pregnancy and provides insights on the dependence of the fetus on the maternal hormone production.

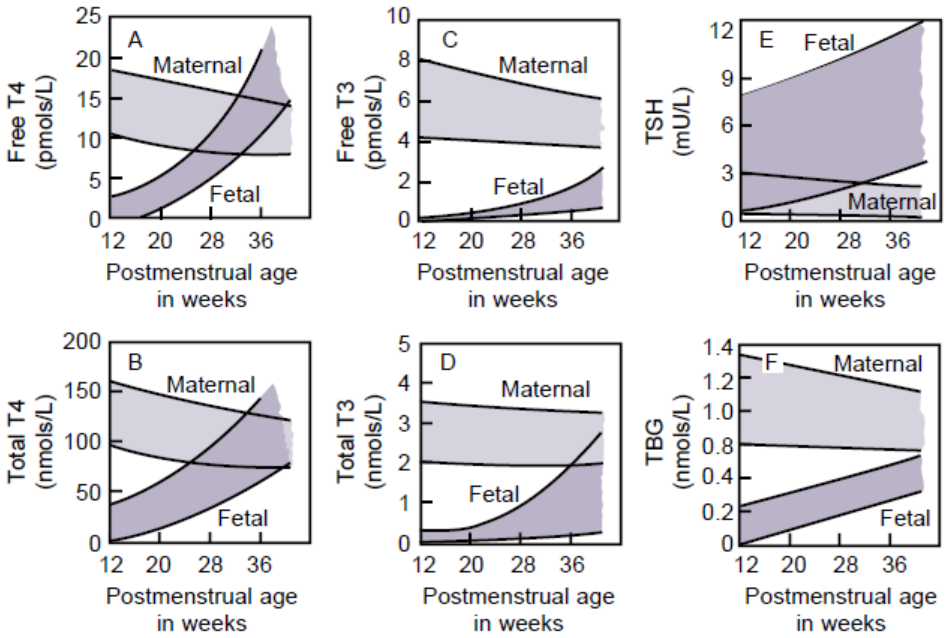

Figure 9. Figure summarizing the changes in maternal and fetal thyroid hormone, TSH and TBG circulation, based on measurements in vivo by cordocentesis. The graphs illustrate concentrations from the 12th week of gestation until birth. It is noteworthy that T3 and FT3 levels are low throughout gestation while fetal levels of fT4 and T4 reach maternal values shortly after midgestation. Figure derived from a review study by Escobar and coworkers (de Escobar, Obregon et al. 2004)**.**

In summary, fetal tissues and especially fetal brain depend on thyroid hormones for their proper development and differentiation. The bioavailability of the unbound, active forms of T4 and T3 which are fT4 and fT3 respectively, depends on maternal thyroid hormone levels during the total period of gestation as well as on the production by the fetal thyroid gland that initiates at approximately 18-20 weeks PMA. Thyroid hormone effects to the fetal brain are regulated by deiodinase enzymes (D1, D2 and D3) the activity of which contributes to the spatial and temporal distribution of thyroid hormone action aiding the differentiation of specific cerebral areas and tissues. Such an effect is also mediated by preferential changes in the concentration and sensitivity of nuclear and TR receptors in different fetal tissues. The deiodinase enzymes also ensure, through their activity, that the levels circulating thyroid hormones neither reach levels high enough to induce toxicity, nor fall below a certain threshold. This regulatory capability of deiodinases however extends to a certain degree and in cases of maternal hypothyroidism with T4 decreases beyond a certain threshold, the fetal brain suffers from thyroid hormone deficiency and its proper development is at risk. Thus, prompt management to safeguard maternal

thyroid activity throughout gestation is often essential for a proper outcome for both the fetus and the mother.

9. THYROID DISORDERS DURING PREGNANCY

9.1. Thyroid autoimmunity and its effects on pregnancy

Various studies have confirmed a relationship between thyroid autoimmunity and pregnancy. Such studies describe an association of the alterations in autoimmunity observed during pregnancy, and the effects that thyroid autoimmunity and especially anti-thyroid peroxidase (TPO) antibody positivity have on the course and outcome of gestation.

The presence of thyroid autoimmunity despite normal thyroid hormone levels involves a spectrum of disorders commonly termed: "*Euthyroid autoimmune thyroid disorders (AITD)*", and has been associated with pregnancy complications such as spontaneous abortion and premature delivery (Vanderpump, Tunbridge et al. 1995, Glinoer 1997). An approximate 10% of the general U.S. population has been found positive for anti-thyroglobulin antibodies and anti-TPO antibodies and this percentage is higher for women in older age (Klein, Haddow et al. 1991, Glinoer 1997). A study by Glinoer et al reports a gradual deterioration of thyroid function during gestation in women with AITD and a high risk of progression to hypothyroidism that could be predicted by measurements of TSH levels and TPO-Ab titers in the first trimester (Glinoer, Lemone 1992, Glinoer 1997). It has been proposed that thyroid immunity is as an independent marker of at-risk pregnancy, with a relative risk of miscarriage being 2 to 4-fold in women with asymptomatic AITD. In addition to the latter, thyroid autoimmunity has been linked to increases in the risk of recurrent miscarriage and miscarriage in women undergoing assistive reproductive technology (Kutteh, Schoolcraft et al. 1999, Muller, Verhoeff et al. 1999, Negro, Schwartz et al. 2010). Furthermore, pregnant women with thyroid antibodies exhibit higher rates of thyroiditis (Marqusee, Hill et al. 1997) and postpartum thyroiditis (Pearce, Farwell et al. 2003) as well as obstetric complications such as placental abruption (Abbassi-Ghanavati, Casey et al. 2010) and premature delivery (Negro, Schwartz et al. 2010). Based on the above data, the authors of the aforementioned studies, among other researchers, suggest that a screening of women with AITD is essential to prevent adverse outcomes during pregnancy (Vanderpump, Tunbridge et al. 1995).

9.1. Pregnancy and Immune System Adaptation

The correlation of thyroid antibody positivity with adverse outcomes during gestation in otherwise healthy women with AITD is very interesting, however the investigation of the course of thyroid autoimmune disease in women that become pregnant has also led to some exciting observations. Form an immunological point of view, the maternal immune system undergoes various adjustments in order to tolerate the fetus, similar to the desired immune tolerance required by a host that receives a transplant. In this respect, the CD4+CD25+ regulatory T cells of the immune system (Treg) shift the balance of Th1/Th2 immunity slightly towards Th2. Since Th-1 immune activity relates to cellular immunity and Th-2 relates to humoral immunity respectively, Treg cells ensure in this manner that the effects of cellular immunity will lessen during pregnancy, since they could jeopardize the survival of the fetus (Mjosberg, Berg et al. 2007). These regulatory adjustments in the immune system would be expected to ameliorate or deteriorate some any pre-existing autoimmune disorders of the mother-host. Indeed, as research shows, the decrease in Th-1 activity leads to an amelioration of pre-existing autoimmune disorders such as multiple sclerosis, autoimmune thyroiditis and rheumatoid arthritis (Adams Waldorf, Nelson 2008). The levels of thyroid autoantibodies, present in Hashimoto's thyroiditis and Grave's disease seem to decrease during gestation and return to their pre-pregnancy values postpartum (Weetman 2011). The findings of current research on the pathophysiology behind autoimmune disorders during pregnancy have provided valuable insights towards the management of disorders such as Grave's disease and Hashimoto's thyroiditis during this period.

9.2. Grave's Disease

Grave's disease is the most common cause of hyperthyroidism and is responsible for 85-90% cases of hyperthyroidism during pregnancy (Galofre, Davies 2009). The pathophysiology of Grave's disease (GD) mainly involves the presence of specific antibodies, called TSH receptor antibodies (TRAbs) that mimic the stimulating effect of TSH on the thyroid gland. The immunosuppression observed during pregnancy, leads to a decrease of TRAb levels in the bloodstream, leading to a protecting effect for both the mother and the fetus. Researchers have observed that the development or relapse of GD is rare during the first trimester of pregnancy, while women with GD during pregnancy exhibit an improvement of the disease during the second trimester (Chan, Mandel 2007, Amino, Izumi et al. 2003). The opposite is

observed postpartum, with 84% of patients with GD experiencing a recurrence of the disease postpartum in contrast to 56% of non-pregnant women (Amino, Izumi et al. 2003).

9.3. Hashimoto's Thyroiditis

Hashimoto's thyroiditis (HT), is another common autoimmune disorder of the thyroid gland, with a prevalence of 18% in the general population (Hollowell, Staehling et al. 2002). Although HT's effects on the thyroid gland are mainly cell-mediated, the disease is accompanied by the presence of anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (TgAb) antibodies in 80-90% of cases respectively (Zaletel, Krhin et al. 2010, Zaletel 2007). Similarly to GD, the antibodies implicated in HT pathology decline during gestation and exhibit their lowest values during the third trimester, while they seem to return to their previous values 12 month postpartum (Smyth, Wijeyaratne et al. 2005, Feki, Omar et al. 2008).

9.4. Postpartum Thyroiditis

The effects that the cessation of the pregnancy-related immune-regulatory activity has on thyroid disorders are most evident in the case of postpartum thyroiditis (PPT). As the term suggests, PT refers to thyroid dysfunction immediately or within a year after the end of gestation. The disorder is characterized by a hyperthyroid phase that does not always occur and presents at about 1-6 months postpartum and lasts for 1-2 months, followed by a hypothyroid phase. The latter occurs 3 to 8 months after delivery and can be transient or permanent (in approcimately 30% of cases) if lasting more than 1 year postpartum (Abalovich, Amino et al. 2007, Kennedy, Malabu et al. 2010, Zaletel, Krhin et al. 2010). The pathophysiology behind the disorder, seems to involve an increase in anti-thyroid autoimmune activity after delivery or miscarriage, that either leads to the emergence of a previously non-existent thyroid disorder, or to the advance of such a disorder from subclinical to clinically obvious levels. It has been shown that the prevalence of PPT is higher in TPOAb positive women (40-60%) and TPOAb patients are more susceptible to hyperthyroidism or permanent hypothyroidism due to PPT (Abalovich, Amino et al. 2007). Hypothyroidism is most probably caused by the destruction of thyroid tissue from the autoimmune activity and as previously explained, is a typical example of the cessation or even reversal of the thyroid-protecting immune regulatory effects of pregnancy in the maternal organism.

The adaptation of the immune system during pregnancy as reflected by Treg cell function, thyroid autoantibody levels and thyroid function in women with autoimmune disorders is illustrated in **figure 10**.

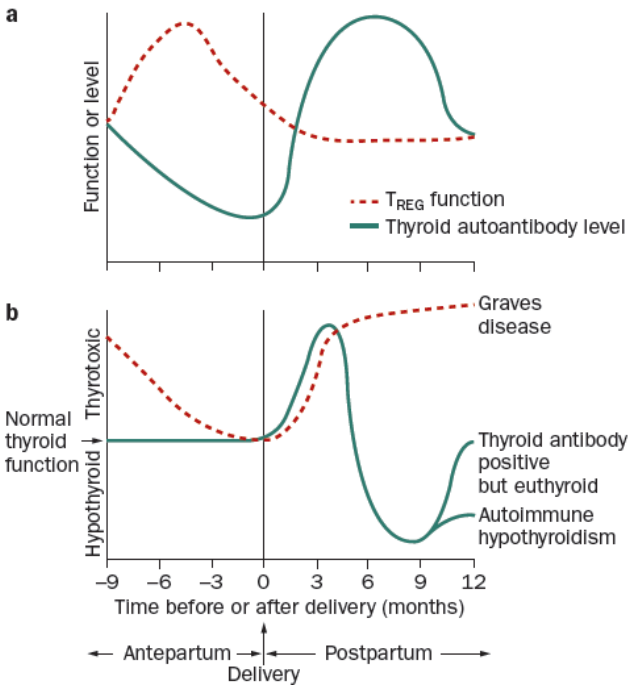

Figure 10. Diagrams illustrating: a) The course of thyroid autoantibody levels and T regulatory cells (Treg) function and b) thyroid function in women with autoimmune thyroid disease during gestation and postpartum. Figure derived from a review article on pregnancy and immunity by Weetman and coworkers (Weetman 2010).

9.2. Thyroid Disorders and their effects on thyroid status: Hypothyroidism and Hyperthyroidism during gestation

The knowledge established on hypothyroidism and hyperthyroidism for the general population provides valuable insights on this matter, however thyroid disease during pregnancy is more complicated in aspects of pathophysiology, diagnosis and treatment. Since a discussion on autoimmune disorders such as Grave's disease and Hashimoto's thyroiditis has already taken place, emphasis will be given on the effects of the hypothyroid or hyperthyroid status on pregnancy outcome.

9.5. Overt and Subclinical Hypothyroidism during Pregnancy

Studies report that a 0.3-0.7% of pregnant women exhibit established hypothyroidism in contrast to a 0.6-1.4% of women in the general population (Gaberscek, Zaletel 2011, van den Boogaard, Vissenberg et al. 2011). This lower

prevalence in pregnant women could be attributed to the fact that hypothyroidism predetermines the odds of a pregnancy in the first place, due to its effects on fertility. Studies have revealed that primary ovulatory infertility risk is 2-fold in women that require thyroid hormone treatment (Grodstein, Goldman et al. 1993, Roti, Minelli et al. 1996). Besides its lower percentage, hypothyroidism does affect pregnant women, either in its overt or in its subclinical form and poses health risk for the mother and the fetus. Some studies report an incidence as high as 2.5% of hypothyroidism in pregnancy (Lazarus 2002). As in the general population, a great number of cases of hypothyroidism during pregnancy are attributed to Hashimoto's thyroiditis, an autoimmune disorder with prevalence as high as 18% in the general population (Hollowell, Staehling et al. 2002). A summary of the causes of hypothyroidism during pregnancy in order of incidence is provided in **Table 2**. Prior to any discussion on hypothyroidism during gestation, some basic concepts on hypothyroidism should be introduced.

Causes of Hypothyroidism in Pregnancy in order of frequency
Hashimoto disease
Postthyroid ablation/removal
Iodine deficiency
Primary atrophic hypothyroidism
Infiltrative disease (e.g., sarcoid, amyloidosis)
TSH-dependent hypothyroidism

Table 2. A summary of the most frequent causes of hypothyroidism during pregnancy. As evident, autoimmunity, iatrogenic causes and iodine deficiency are on the top of the etiologic factors pregnant women. Modified from the review of Fantz and coworkers (Fantz, Dagogo-Jack et al. 1999).

Overt hypothyroidism is defined as the decrease in thyroid activity marked by an elevated TSH and low serum fT4, whilst an increased TSH with serum fT4 values in the normal or the lower normal range characterizes subclinical hypothyroidism.

Research shows that subclinical hypothyroidism is more frequent in pregnant women with type I diabetes (Jovanovic-Peterson, Peterson 1988) and 5-fold more frequent in women with positive thyroid antibodies than in controls (Klein, Haddow et al. 1991). Both overt and subclinical hypothyroidism has been associated with adverse obstetric outcomes by various researchers and a summary of these is provided in a recent meta-analysis by Bogaard and coworkers (van den Boogaard, Vissenberg et al. 2011). Allan W. et al have reported a 4-fold higher rate of fetal death in mothers who

had high TSH values and thus classified as hypothyroid (Allan, Haddow et al. 2000), while pregnancy loss was also observed by other researchers (Abalovich, Gutierrez et al. 2002, Negro, Schwartz et al. 2010). Moreover, other pregnancy complications have been reported in higher rates in hypothyroid women including gestational hypertension (eclampsia, preeclampsia and pregnancy-induced hypertension) (Leung, Millar et al. 1993), placental abruption (Casey, Dashe et al. 2005) and breech delivery (Kuppens, Kooistra et al. 2010).

The maternal complications reported introduce a higher risk of developing postpartum thyroiditis after pregnancy and a long-term risk of developing hypothyroidism later in a woman's life, when hypothyroidism is present during gestation (Vanderpump, Tunbridge et al. 1995). Pregnancy related complications that involve the fetus include fetal death (Allan, Haddow et al. 2000, Ashoor, Maiz et al. 2010) very preterm birth (Stagnaro-Green, Chen et al. 2005) low birth weight (Leung, Millar et al. 1993) and complications possibly associated with preterm delivery such as respiratory distress syndrome and admission to the intensive care unit (Casey, Dashe et al. 2005). Casey and colleagues have reported that such complications have a 2-3 fold higher prevalence in hypothyroid women compared to euthyroid (Casey, Dashe et al. 2005). These results have been confirmed by many studies, however with confounding results for some of the complications especially in the case of mild hypothyroidism (Cleary-Goldman, Malone et al. 2008, Wang, Teng et al. 2012). It is imperative to mention, that the above complications involve the gestation period and pregnancy outcome and are an addition to the aforementioned adverse effects on fetal development, in case hypothyroidism involves low T4 and remains untreated through gestation.

According to the above findings on the effects of hypothyroidism during pregnancy it has been suggested that pregnant women should be screened or at least tested for markers predicting thyroid pathology early in pregnancy. A consensus on whether screening is necessary has not been reached however; The American Association of Clinical Endocrinologists recommends TSH screening in every pregnancy while the American College of Obstetricians and Gynecologists (ACOG) is against it and suggests aggressive case-finding but not screening (Baskin, Cobin et al. 2002, Negro, Schwartz et al. 2010).

9.6. Hyperthyroidism during Pregnancy

The term hyperthyroidism refers to an overactive thyroid gland and a subsequent increase in the levels of thyroid hormones produced in the body. Hyperthyroidism can affect both the mother and the fetus and is respectively called maternal or fetal, although it derives from common pathophysiologic mechanisms both in the mother and the fetus. As explained earlier, the fetal thyroid initiates its function after 20 weeks of gestation and is regulated via the hypothalamic-pituitary axis, and until this period the fetus depends on the transplacental passage of maternal thyroid hormones. The passage of TSH receptor antibodies (TRAbs) through the placenta does not occur until the second half of gestation and therefore maternal hyperthyroidism that is autoimmune in origin does not have implication on the fetus until this period (Rivkees, Mandel 2011). A summary of the causes of hyperthyroidism during pregnancy is provided in **Table 3**.

Causes of hyperthyroidism in pregnancy in order of frequency
Graves' disease (85–90% of all cases)
Sub-acute thyroiditis
Toxic multinodular goiter
Toxic adenoma
TSH-dependent thyrotoxicosis
Exogenous T3 or T4
Iodine-induced hyperthyroidism
Pregnancy-specific associations
Hyperemesis gravidarum
Hydatidiform mole

Table 3. A summary of the most frequent causes of hyperthyroidism during pregnancy. As evident, Grave's disease, is responsible for the majority of hyperthyroidism cases during pregnancy. Modified from the review of Fantz and coworkers (Fantz, Dagogo-Jack et al. 1999).

The diagnosis of maternal hyperthyroidism is based on measurements of elevated total T4 and estimated T4 levels and decreased TSH levels in relation to the expected for the gestational age and may include the presence of TRAbs (Chan, Mandel 2007, Laurberg, Bournaud et al. 2009). The causes of hyperthyroidism include postpartum thyroiditis, toxic thyroid nodules and Grave's disease while thyrotoxicosis can also be caused during gestation in the presence of hydatidiform moles and choriocarcinoma due to the excessive release of hCG. Epidimiological data reveal an incidence of 40 to 50 cases per 100,000 women in childbearing age, while Grave's

disease occurs in 1 per 500 to 1 per 1000 pregnancies (Hall 1995, Lazarus 2002). Grave's disease is therefore responsible for the majority of hyperthyroidism cases during pregnancy, as high as 85-90% of them according to recent findings (Galofre, Davies 2009).

While hyperthyroidism persists or worsens during pregnancy and should be carefully monitored, a similar condition called gestational thyrotoxiosis is temporary and usually remits as the pregnancy advance (Chan, Mandel 2007). The latter reflects an increase in thyroid hormone production due to the effects of hCG and is a finding expected as part of the alterations in thyroid physiology during gestation (Glinoer, De Nayer et al. 1993). Another characteristic of gestational thyrotoxicosis that aid it's diagnosis is the fact that it usually presents between 5 and 15 weeks of gestation and then remits and that it is not associated with goiter and does not involve the presence of TRAbs (Chan, Mandel 2007). It has been reported that there exists an association of gestational thyrotoxicosis with hyperemesis gravidarum, a condition that involves excessive vomiting and requires caution for electrolyte disturbances during gestation (Goodwin, Montoro et al. 1992, Kimura, Amino et al. 1993, Hershman 2004).

In every case of verified maternal hyperthyroidism, caution is needed to prevent its effects on the progression of gestation and fetal development, as it is associated with maternal hypertension and thyroid storm, increased risk of miscarriage, premature birth, fetal demise and growth retardation (Luton, Le Gac et al. 2005).

The fetus can also experience increases in thyroid hormone levels that lead to hyperthyroidism, a disorder associated with maternal hyperthyroidism. In the presence of active or past maternal Grave's disease, the fetus should be closely monitored for fetal hyperthyroidism, since TRAbs can be transferred transplacentally from the mother to the fetus during the second half of gestation. The risk for the latter is proportional to the elevation of TRAb levels (Luton, Le Gac et al. 2005, Polak, Legac et al. 2006, Van Vliet, Polak et al. 2008). In case that fetal hyperthyroidism develops, excessive heart rate (>160 heart beats per minute after 20 weeks), fetal goiter and accelerated maturation of the femoral ossification center are signs that can suggest its presence and provide useful indications of fetal hyperthyroidism for the clinician (Abalovich, Amino et al. 2007).

9.3. Management of Thyroid Disorders in Pregnancy: Special Considerations

The study of thyroid physiology during pregnancy mainly aims in providing knowledge on which clinicians will be based to promote a healthy environment for both the mother and the fetus throughout this period and prevent any adverse outcomes caused by disturbances in thyroid function. Therefore, it is considered necessary to comment on current trends and considerations concerning the treatment of thyroid disorders during pregnancy. Such treatment poses difficulties due to the mother-fetus interaction that raises the possibility of adverse outcomes caused to the fetus from pharmacological interventions during gestation.

9.4. Treatment of Hyperthyroidism During Gestation

Hyperthyroidism treatment in adults is largely based on the antithyroid drug methimazole (MMI) that is used as the first-line drug for most patients (Rivkees 2006). Another common substance prescribed to hyperthyroid patients is propylthiouracil (PTU), a thiouracil-derived drug that was initially used as the first line drug for adults and children but subsequently restricted to the treatment of hyperthyroidism during pregnancy due to the risk for liver injury and even death (Abalovich, Amino et al. 2007, Chan, Mandel 2007, Chattaway, Klepser 2007).

Both substances have some potential interactions on the fetus and the mother that need to be weighted carefully before proceeding to treatment. MMI has been associated with a 17-fold greater risk for the development of choanal atresia in offspring than in the general population with a slightly increased risk of scalp defects (aplasia cutis) and with a risk of fetal malformations according to several studies (Momotani, Ito et al. 1984, Barbero, Valdez et al. 2008). PTU on the other hand has been associated with an increased risk of maternal and fetal liver failure and death during pregnancy (Morris, Goldstein et al. 1989). A combination of the two drugs covering different periods of gestation has proved useful in limiting their adverse effects. It is recommended by various authors that PTU use is limited to the first trimester of gestation and MMI is prescribed afterwards, with a decrease of the prescribed dose during the progression of gestation and its discontinuation when efficable, in approximately 30% of women (Abalovich, Amino et al. 2007, Chan, Mandel 2007, Chattaway, Klepser 2007). Following the use of antithyroid drugs during pregnancy, thyroid function tests are recommended within 48 h after birth and

every 4-7 days for two weeks in order to diagnose potential neonatal hyperthyroidism early. This recommendation is based on the fact that antithyroidal drugs cross the placenta and prevent the manifestation of fetal hyperthyroidism in the presence of circulating TRAbs; this effect ceases postgestationally and can allow thyroid hyperactivation from TRAbs that are yet to be cleared from the fetal circulation (Zimmerman 1999). In case neonatal hyperthyroidism does occur, it is essential that the neonate is closely monitored for this life-threatening condition. Treatment incudes MMI and β blockers and usually lasts for 2-3 months until the TRAbs are cleared from the fetal circulation (Zimmerman 1999).

9.5. Treatment of Hypothyroidism During Gestation

The ideal goal of management for hyperthyroid patients is to achieve euthyroidal function during pregnancy preferably by treating hypethyroidism in advance. Therefore, it is recommended that women with Greave's disease that express the desire to become pregnant be treated with radioactive iodine or surgery prior to pregnancy in order to prevent active hyperthyroidism during gestation. Such therapy is contraindicated during pregnancy due to the accumulation of iodine by the fetal thyroid gland and the subsequent risk of thyroid hypothyroidism and thyroid cancer (Fisher 1997, Chan, Mandel 2007). In order to prevent inadvertent treatment during pregnancy, it is recommended that women with Grave's disease scheduled to undertake iodine treatment be tested for pregnancy within 48 hours of treatment. Moreover, patients treated with iodine are advised to avoid pregnancy for aperiod of 6 months following treatment (Stoffer, Hamburger 1976).

In contrast to hyperthyroidism, treatment for hypothyroidism does not rise any risks for the fetus. However, special consideration and adjustments in treatment have to be made during pregnancy. Levothyroxine, the standard medicine for the treatment of hypothyroidism has to be adjusted in higher doses, requiring a dose increase of 30%-60% in women with autoimmune hypothyroidism and 50%-100% in athyreotic women treated with surgery or radioactive iodine in the past (Mandel, Larsen et al. 1990, Alexander, Marqusee et al. 2004). An increase in the dose of levothyroxine is also required in women taking calcium or iron supplements, or soy products. As for the effects of treatment in the course of pregnancy, one recent study suggests that levothyroxine treatment may attenuate the risk of miscarriage and premature delivery

in TPO-positive women with normal thyroid function, a finding that is expected to be replicated by further studies (Pearce, Farwell et al. 2003).

10. SUMMARY-CONCLUSIONS

Thyroid hormones are vital for the homeostasis of the human body, and during pregnancy they hold a key role in the physiology of both the mother and the fetus as well as a role in fetal development. The production of thyroid hormones, T3 and T4, is achieved by the thyroid gland and is mediated by a complex network of regulatory mechanisms. Between the initiatory stage of iodine uptake and thyroid hormone synthesis and the final end point of thyroid hormone actions in the peripheral tissues, a vast number of biochemical reactions take place, involving a vast number of regulatory pathways in the process. Euthyroidism is characterized by the presence of the required amounts of thyroid hormones in the serum and their availability to the periphery. Briefly, thyroid hormone bioavailability in peripheral tissues depends on various regulatory mechanisms that are governed by some central regulatory "nodes" of interactions. The availability of iodine which is the basic element for hormone production is the first and very important step for achieving euthyroidism. The sequestration of iodine for hormone production is based on the activity of the thyroid gland that is determined through the hypothalamus-pituitary-thyroid axis, while the efficiency of this activity is also based on the presence of a normal, active thyroid gland. Furthermore, the availability of T4 and T3 in the periphery is managed by their biochemical metabolism by certain enzymes called deiodinases. The complexity of these regulatory mechanisms can explain the variety of disorders affecting thyroid hormone production and availability in individual organisms.

Besides the aforementioned complexity of thyroid physiology, the period of pregnancy gives rise to even greater complexities and the achievement of euthyroid status during gestation often proves challenging. Throughout gestation, the thyroid gland is challenged to achieve higher levels of activity and thyroid production. This is marked by an increase in T3 and in the free form of T4, fT4 during the first trimester, accompanied by a decrease in TSH which is explained by the thyrotrophic action of a hormone characteristic of pregnancy, hCG. These events raise the needs in iodine for the mother which if not adequately met, can lead to the appearance of hypothyroidism. Furthermore, previous thyroid disorders of the mother can affect the course of gestation and have been associated with adverse outcomes for both the mother and the fetus.

Such complications have been described for the mother and include among other, gestational hypertension, placental abruption and preterm delivery, while the establishment of abnormal thyroid physiology postpartum has also been described. Autoimmune thyroid disorders such as Grave's disease and Hashimoto thyroiditis are highly important during this period and the fluctuations in maternal autoimmunity due to gestation can affect their course and their effects on the mother and the fetus. The management of such disorders is vital for ensuring euthyroid status during pregnancy and the goal of physicians is to provide treatment for thyroid disorders prior to gestation if possible. Screening for maternal thyroid disorders during gestation has been suggested by various researchers and is already provided in many centers but is not yet widely accepted.

Thyroid function is not only vital for the maternal organism but for the fetus as well. The bioavailability of thyroid hormones in the fetal organism greatly depends on their production and distribution through the maternal circulation as well as on their metabolism by deiodinase enzymes in the fetal tissues. After the onset of fetal thyroid function at about 18-20 weeks of gestation this dependence is less obvious but still exists. Research has proved an association between fetal development and normal thyroid status as well as between thyroid disorders and adverse outcomes for the fetus such as low birth weight or even fetal death. The management of thyroid disorders is further complicated by the effects that treatment regiments for such disorders have on the fetus. Studies have proved an association between certain treatments such as MMI and PTU and abnormalities of the fetus, while the use of treatments such as radioactive iodine is contraindicated during gestation.

The establishment of a normal thyroid status during gestation is of great importance for the normal course of gestation for both the mother and the fetus. Thyroid physiology and pathophysiology are governed by even more complex mechanisms during this period and their proper understanding is vital in employing the optimum screening and treatment strategies. Research has led to some exciting discoveries on the subject, but certain aspects of thyroid physiology and its effects during pregnancy remain unclear. Further studies are expected to expand our knowledge on this very important chapter of physiology during gestation and to provide solutions on still existing controversies such as the employment of screening tests in pregnant women and the management of those diagnosed with thyroid disorders.

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